MORPHOLOGY AND EMBRYOGENY OF THE BALD CYPRESS, *TAXODIUM DISTICHUM* (L.) RICH.

BY

MARGARET KAEISER

B. S., University of Oklahoma, 1934

M. S., University of Oklahoma, 1936

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1940

URBANA, ILLINOIS
1940

MORPHOLOGY AND EMBRYOGENY OF THE BALD CYPRESS, *TAXODIUM DISTICHUM* (L.) RICH.

BY

MARGARET KAEISER

B. S., University of Oklahoma, 1934

M. S., University of Oklahoma, 1936

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1940

URBANA, ILLINOIS
1940

ACKNOWLEDGEMENTS

The writer thanks Dr. J. T. Buchholz for having suggested this problem and for his stimulating interest and advice which have been a constant source of encouragement. Acknowledgment is also made to Dr. Delzie Damaree for his kindness in supplying material for the investigation.

MORPHOLOGY AND EMBRYOGENY OF THE BALD CYPRESS, *TAXODIUM DISTICHUM* (L.) RICH.

I. Introduction

The nomenclature, geographic distribution and history of investigation concerning the general morphology and embryogeny are reviewed in this study. Additional information is given on the morphology and embryogeny of this species, together with a comparison of *Taxodium* with other members of the Taxodiaceae of Pilger.[1] The genus is endemic in North America. Since the work of Coker[2,3] and of Arnoldi[4] on the gametophytes and embryos of the species, Buchholz[5] has furnished a method of dissection, staining, and mounting of conifer embryos which offers a means of more accurately determining their structure and development. There have been, likewise, additional contributions on the morphology and embryology of other genera of the Taxodiaceae of comparative value, and particularly a clarification in embryological terminology in conifers by Buchholz.[6,7] The most recent, although quite brief account of the embryo of *Taxodium* is that of Kapfer.[8]

Coulter[9] had previously reported on the histology of the leaf. Korschelt,[10] Groom,[11] and more recently Cross[12] have dealt with the apical meristems of the shoots. Velenovský[13] reported on the unique sympodial type of branching of the permanent shoots. Bernard,[14] Doak,[15] and Cross[16] have investigated the general morphology of the deciduous shoot. Dluhosch[17] and Hirmer[18] have dealt with the male and female cone structures, respectively. An early report was made by Braun[19] on an abnormal female cone. Radais[20] in 1894 gave an extensive account of the comparative anatomy of the female cones in the Coniferae, including *Taxodium*. Aase[21] mentioned the similarity of the female cone of *Taxodium* to that of *Cryptomeria*. An early report on the "knees" was made by Shaler.[22] Peirce[23] has studied the anatomy of the mature wood. Moon[24] reported on the vascular anatomy of the seedling. Coker and Sax and Sax[25] determined the chromosome number. Thomson[26] measured the megaspore membrane in the mature condition. Von Schrenk[27] first described in detail a disease of the plant known as "peckiness".

II. Materials and Methods

Specimens were collected from various locations in Arkansas and sent both in living condition and preserved in formalin-acetic-alcohol. These were supplemented by collections from one tree growing on the University of Illinois campus. Fixatives included those of formalin-acetic-alcohol for ordinary histological observations; Nawaschin's, Thomas's, or Flemming's solutions were used whenever possible for observations of a more cytological nature. Miscrotome sections of material prepared by the paraffin technique were used for the study of staminate and ovulate cones, male and female gametophytes, fertilization, proembryos, later embryonic development, and for seedlings. Most satisfactory combinations of stains were those of Haidenhain's haematoxylin followed by Orange G in clove oil, and Flemming's Triple. The Buchholz[5] method of dissection, staining, and mounting was followed for early stages in embryogeny. Some aceto-carmine preparations were made of stages in microsporogenesis. Seedlings were killed and fixed in formalin-acetic-alcohol, made into serial sections by the usual paraffin technique, and stained with safranin and fast green. Others, after killing and fixing in the same manner, were cleared in lactic acid. Both methods were used in the study of the transitional anatomy. Hannig's[28] reagent was employed to test the chemical nature of the contents of the secretory elements in the mature embryos and in the seedlings.

III. Investigation

The male cones occur on both simple and compound branches, and occasionally in association with female cones. The scales are arranged spirally. As many as twenty fertile microsporophylls per cone were observed. Nine sporangia per microsporophyll was the maximum found from numerous dissections. This is the largest number reported for any member of the Taxodiaceae. The sporangia are borne abaxially at the proximal portion of the scale and occur in two rows. The origin of the sporogenous and tapetal tissue has been described by Coker. Comparisons made by the writer from reproductions of microsporophylls of seven genera of the Taxodiaceae all drawn to the same scale by

Dluhosch, and from preserved specimens of *Sciadopitys, Sequoiadendron,*[29] *Sequoia, Cunninghamia, Cryptomeria,* and *Taxodium* show that there is no corresponding increase in spore output with the increase in number of sporangia in *Taxodium* when compared with related genera. Spore capacity per sporophyll was found to be highest in *Sequoia,* with *Sciadopitys, Athrotaxis, Cryptomeria, Cunninghamia, Sequoiadendron,* and *Taxodium* in decreasing order. *Taxodium* represents an extreme case in the proportionately small amount of tissue which is given over to spore formation. The study of the details of microsporogenesis and the male gametophyte in general agree with Coker's earlier report. The writer counted eleven pairs of chromosomes, the number reported by Coker and Sax and Sax, and one which is probably the basic number for the family with the exception of the polyploid *Sequoia.* The presence of the papilla in the mature pollen grain is of interest because this structure, although present among investigated members of the Taxodiaceae (with the exception of *Sciadopitys*[30] where a papilla is lacking), is lacking in the Cupressaceae. Pollination occurred February 17, 1939 in Arkansas. Although pollination droplets were not observed in the Arkansas material, they were seen by the writer by April 1, 1939 in cones from the Urbana, Illinois tree.

The outstanding features of the female cone structure in *Taxodium* are: (1) the delay in development of the scale; (2) the greatly developed lobed scale in the mature cone; (3) considerable parenchymatous coalescence of bract and scale in the mature cone exceeding the level of insertion of the ovules; (4) the similarity of this scale anatomically to the ovuliferous scale of the pines; (5) the close resemblance of the cone to that of *Cryptomeria,* although there is an even more extended growth of scale in *Taxodium;* (6) the insertion of the ovules near to the axis of the cone; (7) the presence of two erect ovules per bract; (8) and the presence of secretory canals particularly conspicuous in the region subtending the vascular system of the bract.

Details of megasporogenesis have been reported by Coker and the writer's findings agree with his account. All of the stages of fertilization were not available in the writer's material. The size of the male nucleus increases rapidly after contact with the female nucleus is made, as Coker described, and the appear-

ance of the fusing nuclei agrees with his account. A pronounced darkly staining area was seen neither around the central nucleus nor the egg and ventral canal nuclei (before fertilization, and so long as the large central vacuole of the archegonium was present these areas were not discovered. The large vacuole so conspicuous before fertilization disappears. Egg nuclei of archegonia adjacent to archegonia containing fusing nuclei also failed to show these heavily stained areas. The need for intensive cytological study concerning the origin of the cytoplasmic sheath surrounding the fusing nuclei is indicated. There is the possibility that the dehydration taking place during this period when the large central vacuole disappears may in some measure account for the starch accumulation that is claimed to occur. The writer's material does not preclude the possibility of at least some of the male cytoplasm contributing to this sheath. However, starch grains could not be demonstrated by the use of a polarizing microscope under 720 times magnification, either in fusion stages or in the proembryos.

The male and female nuclei sink to the bottom of the archegonium before the first division takes place. Three nuclear divisions occur before wall formation takes place. The common arrangement of nuclei observed in the eight nucleated stage is two nuclei below and six above, although Coker reported seven above and one below, or five above and the remaining three near to the base of the archegonium in some cases. These variations were also found by the writer. The morphologically upper group of cells is open. Following the eight celled stage there are nuclear divisions in both the upper and lower groups of cells, the spindle axes in the upper being usually parallel to the longitudinal axis of the archegonium, whereas those in the lower group are at right angles to it. The resulting sixteen celled proembryo consists of three groups of cells, the uppermost of which arranged in a tier has no end walls; the middle row of cells forming another group; and the lowermost group not distinctly arranged in a tier. The morphologically upper tier of six cells may be called the *open tier* rather than the rosette tier as Coker originally designated. This open tier is structurally similar to the relict tier of Schopf's[31] terminology and would not be confusing with the open rosette tier, a term which is employed by

Mathews[32] in connection with *Juniperus virginiana*. The middle tier of six cells upon elongation forms the first suspensor system of the embryo, and, because this suspenosr originates from a tier of cells organized in the proembryo and begins to elongate as a unit rather than independently, it is by definition the prosuspensor. The four lowermost cells are the embryo initials. The entire proembryo occupies usually less than one-half of the archegonium at this stage. The elongation of the prosuspensor causing the emergence of the embryo initials marks the end of the proembryo stage. The numbers of nuclei counted in the upper portions of various archegonia during the fusion stages and proembryo formation were from two to five. No mitoses of these nuclei were observed. It is possible that in sectioning, certain nuclei of the jacket cells were displaced, although this does not appear to be the case from numerous observations; it is possible that the ventral canal nucleus may divide as Coker claimed (The "amitotic division" described by him may be a nuclear phenomenon to be associated with its degeneration.); or it is even possible that some of the nuclei brought in by the pollen tube may divide.

The prosuspensor increases greatly in extent and becomes considerably coiled before further division of the embryo initials takes place. Never more than six embryo systems were observed to have emerged from one archegonial complex. An apical cell may persist into the multicellular embryo stage, although no direct proof of its presence could be established in mature or nearly mature embryos. In only one case was an isolated prosuspensor cell observed to have a single elongated cell separating it from its two celled embryo unit. This single cell would be called a primary suspensor. The majority of embryos examined produced the secondary suspensor cells in groups, and only after the embryo had become multicellular. The embryo initial units of a system may remain together for a short time but soon separate from each other probably because of the differences in rate of elongation of the prosuspensor cells. This separation marks the beginning of cleavage polyembryony which appears without exception in all material examined at this stage. Soon after each embryo has become multicellular the initial cells of embryonal tubes may be detected by their

rapid increase in size and large vacuoles. These cells are generally cut off at one level at a time and at a later stage of development constitute the massive secondary suspensor of each embryo. They are comparable to the "supernumerary suspensors" that Coker mentions. There is a slight tendency, however, for these initial cells of embryonal tubes to be formed individually. The dissection technique also revealed the curious behavior of many of the isolated prosuspensor cells. They become multicellular often by internal division, by cutting off additional cells which may in turn elongate somewhat, or in extreme cases by branching without forming cross walls, the tops of the lobes or branches then cutting off small end cells. Similar behavior of prosuspensor cells have been noted in *Cryptomeria japonica* by Buchholz[33] and may be considered as evidence that these prosuspensor cells are potential embryo initials. There is a resemblance here to the rosette cells (completely walled cells) of certain other genera which are capable of forming embryos. The absence of rosette embryos in *Taxodium* is a significant feature of its embryogeny.

The mature embryo is most often slightly curved. Both hypocotyl and cotyledons are elongated structures about equal in length. The calyptroperiblem region is defined by a groove extending around the lower portion of the embryo. Upon comparison with mature embryos of other genera of the Taxodiaceae, that of *Taxodium* shows greatest similarity to *Sequoiadendron*. The calyptroperiblem region of all investigated members is shorter than those found among the Pinaceae. No plumlar primordia were discernible in the mature embryos even from cones after the shattering stage. The shoot apex is a comparatively flattened dome completely hidden by the enveloping cotyledons. Examination of four hundred embryos gave a range of four to eight cotyledons. The mean was 6.09 ± 0.03 cotyledons, and the standard deviation was 0.61 ± 0.02. Only one case of twin embryos was discovered, and they were about one-third matured in size. The order of internal differentiation of the various parts of the embryo agrees with that reported for *Cedrus*.[34] Secretory elements were observable in both the hypodermal layer and in the plerome of the embryo. When freshly dissected embryos were treated with Hannig's solution of 1% chromic acid satur-

ated with copper acetate these elements turned brownish. None showed a characteristic reaction for resin. When tannin is treated with a 1% chromic acid solution a brownish precipitate is formed. Critical examination of young embryos failed to reveal any indication of ontogenetic fusion of cotyledon primordia such as that reported by Buchholz[35] for other genera of conifers.

Cutting tests of seeds from one hundred cones collected November 22, 1939 revealed the following data: Average cone size, 33 mm. in length and 27.7 mm. in width. A total of 1,394 seeds was obtained. Thirty-seven of the one hundred cones showed abnormally developed ovules, totalling one hundred ovules in all. Of the 1,394 seeds seemingly normal in external appearance, only 16.6% yielded good embryos, or 2.3 embryos per cone; the balance were either empty or contained shriveled gametophytes. Only eleven (0.7%) cases of this lot had two good embryos on the same scale; none showed more than one such scale per cone. There were usually two seeds per scale. The yield of 16.6% good embryos is quite low in comparison with about 46% reported by the Thompson Tree Nursery of Jonesboro, Illinois for crops previous to 1939 in southern Illinois.

A detailed study of the transitional anatomy of the seedling revealed that (1) *Taxodium* may be included in Van Tieghem's[36] Type 3; (2) they most generally follow Hill and DeFraine's[37] second variation within this type, in which the seed-leaf-trace is endarch but the rotation of the protoxylem takes place in the hypocotyl; (3) there are present ordinarily "whole cotyledons" of Hill and DeFraine's description (or, more specifically, the Type C cotyledons of Boureau[38] because the transition is completed in the hypocotyl and the cotyledon strands themselves are endarch); (4) the "subsidiary" or Type D cotyledon may occur occasionally; (5) partial fusion of all or only two cotyledons may occur at the bases, simulating cotyledonary tube formation.

IV. Discussion and Conclusion

Noteworthy among the morphological and embryological features of *Taxodium distichum* (L.) Rich. which is endemic to North America are: (1) the sympodial branching of the permanent shoots; (2) its deciduous habit; (3) formation of pneumato-

phores; (4) dwarf shoots; (5) the usual shattering of its female cones (6) containing irregularly triangular woody seeds with reduced wings; (7) the highly resinous woody cone; (8) the spiral arrangement of the cone scales; (9) the comparatively late development of the ovuliferous scale (10) with its subsequent large size in comparison with the bract; (11) the two erect ovules present in the mature bract (12) with their insertion near to the central cone axis; (13) the large number of microsporangia per scale (14) and their arrangement in two definite rows; (15) the extreme reduction in proportionate amount of scale occupied by these structures; (16) the two-nucleated microgametophytes or pollen; (17) pollen with papillae; (18) the occurrence of the cupressinean shaped megagametophyte (19) with an archegonial complex which may vary in arrangement, number, and position of archegonia; (20) the occurrence of only three megaspores; (21) the presence of two equal sized male cells; (22) the presence of "supernumerary" nuclei in the upper portion of archegonia containing developing proembryos; (23) the variation in number of neck cells; (24) the occurrence of the first division of the fusion nucleus at the base of the archegonium; (25) three free nuclear divisions before wall formation in the proembryo; (26) irregular arrangement of the groups of cells in the proembryo; (27) relative thickness of the megaspore membrane; (28) absence of rosette cells or rosette embryos; (29) rare occurrence of a primary suspensor; (30) presence of a well developed pro-suspensor, and a massive secondary suspensor; (31) presence of a short-lived apical cell in early embryonic development; (32) presence of cleavage polyembryony, somewhat restricted in that rosette embryos do not occur; (33) cotyledons more numerous, generally, than in any other member of the Taxodiaceae so far reported.

In comparing *Taxodium* with other members of the Taxodiaceae the writer concludes that it is more similar to *Cryptomeria* on the basis of structure and behavior of proembryos and early embryos, its wood anatomy, and the general structural pattern of the female cone, than to any other genus. *Taxodium* is likewise similar to *Glyptostrobus* in some respects; *Cunninghamia* and *Taiwania* both have anatropous ovules and may be tentatively placed together on the basis of secondary wood anat-

omy as well. It is evident, however, that the position of the last two named genera would be lower phylogenetically than *Cryptomeria, Glyptostrobus* and *Taxodium. Sequoiadendron, Athrotaxis,* and *Sequoia* show close similarities. *Sciadopitys* appears well differentiated from all the rest.

LITERATURE CITED

[1] Pilger, R. Die Natürlichen Pflanzenfamilien. Engler-Prantl. Leipzig. 1926.
[2] Coker, W. C. Johns Hopkins Univ. Circ. 1900.
[3] Coker, W. C. Bot. Gaz. 36: 1-27; 114-140. 1903.
[4] Arnoldi, W. Bull. Soc. Nat. Moscou. N. S. 14: 449-476. 1900.
[5] Buchholz, J. T. Stain Tech. 13: 53-64. 1938.
[6] Buchholz, J. T. Amer. Jour. Bot. 7: 125-145. 1920.
[7] Buchholz, J. T. Proc. Fourth Internat. Bot. Congr. 1: 359-392. 1929.
[8] Kapfer, E. Abst. of Diss. München. 1935.
[9] Coulter, S. Bot. Gaz. 14: 76-81. 1889.
[10] Korschelt, P. Jahrb. wiss. Bot. 15:642-674. 1884.
[11] Groom, P. Ber. d. deut. Bot. Gesell. 3: 303-312. 1885.
[12] Cross, G. L. Bull. Torr. Bot. Club. 66:431-452. 1939.
[13] Velenovský, J. Vergleichende Morphologie der Pflanzen. Part 1, Prag. 1905.
[14] Bernard, C. Proc. Linn. Soc. N. S. Wales. 51: 114-128. 1926.
[15] Doak, C. C. Univ. Ill. Monograph. 13: 1-106. 1935.
[16] Cross, G. L. Bull. Torr. Bot. Club. 66: 167-172. 1939.
[17] Dluhosch, H. Bibliotheca Botanica Heft 114 (3) Stuttgart. 1937.
[18] Hirmer, M. Bibliotheca Botanica Heft 114 (1) Stuttgart. 1936.
[19] Braun, A. Vortrag auf dem Congres scientifique de France, 10th Session (Strassburg), pp. 171-172. 1842. (Communicated in Braun's Leben von C. Mettenius, p. 335. Berlin, 1882.)
[20] Radais, M. L. Ann. Sci. Nat. Bot. sér. T. VII. 19: 165-368. 1894.
[21] Aase, H. Bot. Gaz. 60: 277-313. 1915.
[22] Shaler, N. S. Sci. 2: 38-40. 1883.
[23] Peirce, A. S. Univ. Ill. Ph.D. Thesis. 1935.
[24] Moon, G. M. Univ. Ill. M.S. Thesis, 1938.
[25] Sax, K. and Sax, Hally J. Jour. Arn. Arb. 14: 356-375. 1933.
[26] Thomson, R. B. Toronto Univ. Studies. Biol. Ser. No. 4, pp. 1-64. 1905.
[27] Von Schrenk, H. 11th Ann. Rpt. of the Mo. Bot. Gard. St. Louis. 1899.
[28] Hannig, E. Zeitsch. für Bot. 14: 385-419. 1922.
[29] Buchholz, J. T. Amer. Jour. Bot. 26: 535-538. 1939.
[30] Kaeiser, M. Trans. Ill. State Acad. Sci. 32:91-93. 1939.
[31] Schopf, J. M. Univ. of Ill. Ph.D. Thesis. 1937.
[32] Mathews, A. C. Jour. Elisha Mitchell Sci. Soc. 55: 7-62. 1939.
[33] Buchholz, J. T. Bot. Gaz. 93:221-226. 1932.
[34] Buchholz, J. T. and Old, E. M. Amer. Jour. Bot. 20: 35-44. 1933.
[35] Buchholz, J. T. Amer. Jour. Bot. 6:106-119. 1919.
[36] Van Tieghem, P. Ann. Sci. Nat. Bot. sér. v^{e}, T. XIII, p. 1. 1871.
[37] Hill, T. G. and DeFraine, E. Ann. Bot. 22: 689-712. 1908.
[38] Boureau, E. Ann. Sci. Bot. sér. 11^{e}, T. I. pp. 1-219. 1939.

VITA

Margaret Kaeiser was born in McAlester, Oklahoma on June 20, 1912. She completed her high school training at McAlester High School in 1929. She received the B. S. degree in 1934 and the M.S. degree in 1936 from the University of Oklahoma. During the summer of 1935 she was enrolled as a student at the Rocky Mountain Summer Camp of the University of Wyoming. From 1937-39 she held assistantships and from 1939-40 a fellowship in botany at the University of Illinois.

She is a member of Phi Sigma, Sigma Xi, Oklahoma Academy of Science, Illinois State Academy of Science, Sigma Delta Epsilon, and The Botanical Society of America.

A paper entitled "Note on Embryo Development in *Hippuris*" was read by her at the 1940 spring meeting of the Illinois State Academy of Science.

Publications

Transitional Phenomena in a Normal Seedling of *Althea rosea* Cav. Proc. Okla. Acad. Sci. 17: 62-65. 1937.

Pollen of Certain Taxodiaceae. Trans. Ill. State Acad. of Sci. 32: 91-93. 1939.

(with J. T. Buchholz) A Statistical Study of Two Variables in the Sequoias-Pollen Grain Size and Cotyledon Number. Amer. Nat. 74: 279-283. 1940.